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TITLE: Recombinant botulinum toxins having a soluble C-terminal portion of a heavy chain, an N-terminal portion of a heavy chain and a light chain

PUBLICATION-DATE: July 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Application 10/729039 is a division-of US application 10/271012, filed October 15, 2002, PENDING
Application 10/271012 is a continuation-of US application 08/704159, filed August 28, 1996, PENDING
Application 08/704159 is a continuation-in-part-of US application 08/405496, filed March 16, 1995, US Patent No. 5919665

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REPRESENTATIVE-FIGURES: NONE

ABSTRACT:

The present invention includes recombinant proteins derived from Clostridium botulinum toxins. In particular, soluble recombinant Clostridium botulinum type A, type B and type E toxin proteins are provided. Methods which allow for the isolation of recombinant proteins free of significant endotoxin contamination are provided. The soluble, endotoxin-free recombinant proteins are used as immunogens for the production of vaccines and antitoxins. These vaccines and antitoxins are useful in the treatment of humans and other animals at risk of intoxication with clostridial toxin.

[0001] This application is a Continuation-In-Part of copending application Ser. No. 08/405,496, filed Mar. 16, 1995.

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US-CL-CURRENT: 435/252.33; 435/254.2, 435/320.1, 435/348, 435/69.3, 530/350, 536/23.7

CLAIMS:

1. A host cell containing a recombinant expression vector, said vector encoding a protein comprising at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

2. The host cell of claim 1, wherein and said host cell is capable of expressing said protein at a level greater than or equal to 5% of the total cellular protein.

3. The host cell of claim 1, wherein and said host cell is capable of expressing said protein as a soluble protein at a level greater than or equal to 0.25% of the total soluble cellular protein.

4. The host cell of claim 1, wherein said host cell is an Escherichia coli cell.

5. The host cell of claim 1, wherein said host cell is an insect cell.

6. The host cell of claim 1, wherein said host cell is a yeast cell.

7. A host cell containing a recombinant expression vector, said vector encoding a fusion protein comprising a non-toxin protein sequence and at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

8. The host cell of claim 7, wherein said portion of said toxin comprises the receptor binding domain.

9. The host cell of claim 7, wherein said non-toxin protein sequence comprises a poly-histidine tract.

10. A vaccine comprising a fusion protein, said fusion protein comprising a non-toxin protein sequence and at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

11. The vaccine of claim 10 further comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of Clostridium botulinum type A toxin.

12. The vaccine of claim 10, wherein said portion of said Clostridium botulinum toxin comprises the

receptor binding domain.

13. The vaccine of claim 10 wherein said non-toxin protein sequence comprises a poly-histidine tract.

14. The vaccine of claim 10, wherein said vaccine is substantially endotoxin-free.

15. A method of generating antibody directed against a Clostridium botulinum toxin comprising: a) providing in any order: i) an antigen comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin, and ii) a host; and b) immunizing said host with said antigen so as to generate an antibody.

16. The method of claim 15, wherein said antigen further comprises a fusion protein comprising a non-toxin protein sequence and at least a portion of Clostridium botulinum type A toxin.

17. The method of claim 15, wherein said portion of said Clostridium botulinum toxin comprises the receptor binding domain.

18. The method of claim 15 wherein said non-toxin protein sequence comprises a poly-histidine tract.

19. The method of claim 15 wherein said host is a mammal.

20. The method of claim 19 wherein said mammal is a human.

21. The method of claim 15 further comprising step c) collecting said antibodies from said host.

22. The method of claim 21 further comprising step d) purifying said antibodies.

23. The antibody raised according to the method of claim 15.

24. The antibody raised according to the method of claim 16.

DOCUMENT-IDENTIFIER: US 20040115215 A1
TITLE: Recombinant botulinum toxins with a soluble C-terminal portion, an N-terminal portion and a light chain

CLAIMS:

1. A host cell containing a recombinant expression vector, said vector encoding a protein comprising at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

7. A host cell containing a recombinant expression vector, said vector encoding a fusion protein comprising a non-toxin protein sequence and at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

8. The host cell of claim 7, wherein said portion of said toxin comprises the receptor binding domain.

10. A vaccine comprising a fusion protein, said fusion protein comprising a non-toxin protein sequence and at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin.

11. The vaccine of claim 10 further comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of Clostridium botulinum type A toxin.

12. The vaccine of claim 10, wherein said portion of said Clostridium botulinum toxin comprises the receptor binding domain.

15. A method of generating antibody directed against a Clostridium botulinum toxin comprising: a) providing in any order: i) an antigen comprising a fusion protein comprising a non-toxin protein sequence and at least a portion of a Clostridium botulinum toxin, said toxin selected from the group consisting of type B toxin and type E toxin, and ii) a host; and b) immunizing said host with said antigen so as to generate an antibody.

16. The method of claim 15, wherein said antigen further comprises a fusion protein comprising a non-toxin protein sequence and at least a portion of Clostridium botulinum type A toxin.

17. The method of claim 15, wherein said portion of said Clostridium botulinum toxin comprises the receptor binding domain.

PGPUB-DOCUMENT-NUMBER: 20020068699
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20020068699 A1

TITLE: Clostridial toxin derivatives and methods for treating pain

PUBLICATION-DATE: June 6, 2002

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Donovan, Stephen	Capistrano Beach	CA	US	

US-CL-CURRENT: 514/12; 530/350

CLAIMS:

I claim:

1. An agent, comprising: (a) a clostridial neurotoxin, or component thereof, attached to; (b) a targeting moiety, wherein the targeting moiety is selected from the group consisting of transmission compounds, and compounds substantially similar to the transmission compounds.

2. The agent of claim 1, wherein the transmission compound can be released by a neuron to transmit or to facilitate the transmission or the generation of a pain signal.

3. The agent of claim 1, wherein the clostridial neurotoxin includes neurotoxins as well as derivatives and fragments of neurotoxins made by a Clostridial beratti, Clostridial butyricum, Clostridial botulinum, or Clostridial tetani bacterium.

4. The agent of claim 1, wherein the clostridial neurotoxin component is derived from botulinum toxin selected from the group consisting of botulinum toxin types A, B, C, D, E, F, G and mixtures thereof.

5. The agent of claim 1, wherein the clostridial neurotoxin component includes at least one of a heavy chain, a fragment of a heavy chain, a light chain and a fragment of a light chain.

6. The agent of claim 5, wherein the fragment of a heavy chain or the light chain includes at least one of a carboxyl end segment and an amine end segment.

7. The agent of claim 4, wherein the clostridial neurotoxin comprises at least a part of a heavy chain and at least a part of a light chain of a clostridial neurotoxin, the heavy chain being derived from one botulinum toxin serotype and the light chain being derived from a different botulinum toxin serotype.

8. The agent of claim 1, wherein the clostridial neurotoxin component comprises a light chain or a fragment of a light chain linked to a heavy chain or to a fragment of a heavy chain by a direct covalent linkage.

9. The agent of claim 1, wherein the clostridial neurotoxin has a light chain or a fragment of a light chain linked to a heavy chain or a fragment of a heavy chain by one or more spacer components.

10. The agent of claim 1, wherein the transmission compound is selected from the group consisting of amino acids, substituted counterparts thereof and mixtures thereof.

11. The agent of claim 10, wherein the transmission compound is selected from a group consisting of glutamates, substituted counterparts thereof and mixtures thereof.

12. The agent of claim 10, wherein the amino acids are linked to form at least one peptide.

13. The agent of claim 12, wherein at least one peptide is a tachykinin.

14. The agent of claim 13, wherein the tachykinin is substance P.

15. The agent of claim 12, wherein at least one peptide is selected from a group consisting of natural precursors of substance P and synthetic precursors of substance P.

16. The agent of claim 12, wherein at least one peptide is selected from the group consisting of fragments of substance P.

17. The agent of claim 12, wherein at least one peptide is selected from the group. consisting of substance P analogues comprising at least one D-amino acid and substance P analogues comprising a disulfide bridge.

18. The agent of claim 1, wherein the clostridial neurotoxin is covalently attached to the targeting moiety.

19. The agent of claim 1, wherein the clostridial neurotoxin is covalently coupled to the targeting moiety through one or more spacer components.

20. The agent of claim 14, wherein the clostridial neurotoxin comprises a light chain linked to a fragment of a heavy chain, wherein the heavy chain is derived from an amine end segment of a heavy chain of a botulinum neurotoxin toxin type A and the targeting moiety comprises Substance P.

21. A method for obtaining an agent for alleviating pain, the method comprising: (a) producing a genetic construct having codes for a clostridial neurotoxin or component thereof selected from the group consisting of a clostridial neurotoxin, a modified clostridial neurotoxin and fragments thereof; (b) incorporating the construct into a host organism; (c) expressing the construct to produce the clostridial neurotoxin component; and (d) covalently attaching the clostridial neurotoxin to a targeting moiety selected from the group consisting of transmission compounds released from neurons in transmitting pain signals and components substantially similar to the transmission compounds.

22. The method of claim 21, wherein the covalent linkage includes one or more spacer components.

23. A method for obtaining an agent for treating pain, the method comprising: (a) producing a genetic construct having codes for (1) a clostridial neurotoxin component selected from a group consisting of clostridial neurotoxin, a modified clostridial neurotoxin and fragments thereof and (2) a targeting moiety selected from the group consisting of transmission compounds released from neurons in transmitting pain signals and components substantially similar to the transmission compounds; (b) incorporating the genetic construct into a host organism; and (c) expressing the genetic construct to obtain a fusion protein comprising the clostridial neurotoxin components covalently coupled to the targeting moiety.

24. The method of claim 23, wherein the genetic construct includes genetic codes that encode for a spacer component between the clostridial neurotoxin component and the targeting moiety.

25. The method of claim 23, wherein the targeting moiety is substance P.

26. An polypeptide agent for alleviating pain, the agent comprising: (a) a first amino acid sequence region comprising: (i) a first domain, the first domain comprising a targeting moiety, the targeting moiety being selected from the group consisting of transmission compounds released from neurons in transmitting pain signals and compounds substantially similar to the transmission compounds; and (ii) a second domain, the second domain comprising a translocation element able to facilitate the transfer of the polypeptide across an endosome membrane, and b) a second amino acid sequence region comprising a therapeutic element having biological activity or therapeutic activity when released into the cytoplasm of a target cell.

27. The polypeptide of claim 26, wherein the second domain of the first amino acid sequence region comprises a clostridial neurotoxin heavy chain or derivative or fragment thereof.

28. The polypeptide of claim 27, wherein the clostridial neurotoxin heavy chain is derived from a Clostridial botulinum neurotoxin type A.

29. The polypeptide of claim 27, wherein the second amino acid sequence region comprises a clostridial neurotoxin light chain.

30. The polypeptide of claim 29, wherein the clostridial neurotoxin light chain is derived from Clostridial botulinum neurotoxin type A.

31. The polypeptide of claim 27, wherein the clostridial neurotoxin heavy chain is derived from Clostridial tetani neurotoxin.

32. The polypeptide of claim 27, wherein the clostridial neurotoxin heavy chain is derived from an organism selected from the group consisting of Clostridial botulinum type B, C, D, E, F and G; Clostridial baratii; and Clostridial butyricum.

33. The polypeptide of claim 26, wherein the first domain comprises a targeting moiety able to bind surface receptors of spinal cord neurons under physiological conditions.

34. The polypeptide of claim 33, wherein the second domain comprises a clostridial neurotoxin light chain.

35. The polypeptide of claim 26, wherein the targeting moiety specifically binds a receptor on a spinal cord dorsal horn neuron.

36. A plasmid encoding a polypeptide that is derived from a clostridial neurotoxin, comprising: a) a first nucleotide sequence region comprising; i) a first portion encoding an amino acid sequence region comprising a targeting moiety that is (1) selected from a group consisting of transmission compounds released from neurons in transmitting pain signals and components substantially similar to the transmission compounds, and (2) able to specifically bind to receptors on cells under physiological conditions; and ii) a second portion encoding an amino acid sequence region comprising a translocation element able to facilitate the transfer of a polypeptide across an endosome membrane; and b) a second nucleotide sequence region encoding an additional amino acid sequence region comprising a therapeutic

element having biological activity when released into the cytoplasm of a target cell, and an origin of replication directing plasmid replication by a host cell.

37. A method of making a polypeptide derived from a clostridial neurotoxin comprising: (a) inserting the plasmid of claim 36 into a suitable host cell, (b) growing the host cell in culture, and (c) permitting the host cell to express the polypeptide from the plasmid.

38. A method for treating pain, the method comprising the step of administration to a human patient of a therapeutically effective amount of an agent, wherein the agent comprises a clostridial neurotoxin component coupled to a targeting moiety selected from a group consisting of transmission compounds released from neurons in transmitting pain signals and components substantially similar to the transmission compounds.

39. The method of claim 38, wherein the clostridial neurotoxin component is derived from botulinum toxin selected from the group consisting of botulinum types A, B, C, D, E, F, G and mixtures thereof.

40. The method of claim 39, wherein the clostridial neurotoxin component comprises a light chain and an amine end segment of a heavy chain.

41. The method of claim 40, wherein the targeting moiety comprises substance P.

42. The method of claim 39, wherein the agent contains botulinum toxin in an amount between about 10.sup.-3 U/kg and about 35 U/kg.

43. The method of claim 39, wherein the agent contains botulinum toxin in an amount between about 1 U/kg and about 10 U/kg.

44. The method of claim 39, wherein the agent contains botulinum toxin in an amount bout 3 U/kg.

45. The method of claim 39, wherein the agent contains botulinum toxin in an amount between about 1 U and about 500 U.

46. The method of claim 39, wherein the agent contains botulinum toxin in an amount between about 10 U and about 300 U.

47. The method of claim 39, wherein the agent contains botulinum toxin in an amount about 70 U.

48. The method of claim 38, wherein the pain alleviating effect persists for from about 2 to about 6 months.

49. The method of claim 38, wherein the agent is administered locally at the periphery.

50. The method of claim 49, wherein the agent is administered intramuscularly.

51. The method of claim 38, wherein the agent is administered intrathecally.

52. The method of claim 38, wherein the agent is administered intrathecally to a cranial region of the central nervous system.

53. The method of claim 38, wherein the agent is administered intrathecally to a cervical region of the

central nervous system.

54. The method of claim 38, wherein the agent is administered intrathecally to a thoracic region of the central nervous system.

55. The method of claim 38, wherein the agent is administered intrathecally to a lumbar region of the central nervous system.

56. The method of claim 38, wherein the agent is administered intrathecally to a sacral region of the central nervous system.

57. The method of claim 38, wherein the administration step includes the steps of: (a) accessing a subarachnoid space of a central nervous system of a mammal, and; (b) injecting the agent into the subarachnoid space.

58. The method of claim 57, wherein the accessing step is carried out by effecting a spinal tap.

59. The method of claim 38, wherein a administration step includes the steps of: (a) catheterization of a subarachnoid space of the central nervous system of a mammal, and; (b) injecting the agent through a catheter inserted by the catheterization step into the subarachnoid space.

60. The method of claim 59, wherein the administration step includes, prior to the injecting step, the step of attaching to or implanting in the mammal an administration means for administering the agent to the central nervous system of the mammal, the administration means comprising a reservoir of the agent, the reservoir being operably connected to a pump means for pumping an aliquot of the agent out of the reservoir and into an end of the catheter in the subarachnoid space.

61. The method of claim 38, wherein the administration step is carried out prior to the onset of a nociceptive event or syndrome experienced by the mammal.

62. The method of claim 61, wherein the administration step is carried out before to about 14 days before the onset of the nociceptive event.

63. The method of claim 38, wherein the administration step is carried out after the onset of a nociceptive event.

64. The method of claim 63, wherein the nociceptive event is a neuropathic pain syndrome.

65. The method of claim 63, wherein the nociceptive event is inflammatory pain.

66. An agent for treating pain, the agent comprising: (a) a botulinum toxin type A proteolytic domain attached to; (b) a botulinum toxin type A translocational domain, and (c) substance P attached to the translocational domain.

Table 2

Proliferative T Cell Response in human vaccines

Stimulating Antigen* (µg/ml)		[^{3}H]TdR incorporation (CPM)+ VK	DS	MJC
Med		486 ± 13	297 ± 73	590 ± 59
PHA		332213 ± 12212	71161 ± 3577	127973 ± 8181
BSA	(5)	453 ± 27	289 ± 52	400 ± 33
BTxd	(5)	64042 ± 14834	60395 ± 3652	259572 ± 19086
	(1)	39538 ± 4428	50724 ± 1775	ND
L	(5)	65830 ± 14521	53928 ± 5995	178867 ± 18003
	(1)	33638 ± 6219	26782 ± 1900	95035 ± 8030
H	(5)	38400 ± 2009	47352 ± 10304	149619 ± 3331
	(1)	14353 ± 4430	20908 ± 7857	60213 ± 1701
L-H_N	(5)	75656 ± 15980	67961 ± 7784	ND
	(1)	32833 ± 4432	8186 ± 3771	ND
P1	(25)	32062 ± 4778	2458 ± 1469	650 ± 62
	(2.5)	14068 ± 6058	1187 ± 851	647 ± 53
	(0.25)	13844 ± 5893	301 ± 40	356 ± 16
P2	(25)	486 ± 127	751 ± 279	1894 ± 1166
	(2.5)	1019 ± 389	384 ± 205	603 ± 144
P3	(25)	811 ± 69	554 ± 139	2102 ± 407
	(2.5)	491 ± 37	256 ± 58	441 ± 60
P4	(2.5)	867 ± 101	315 ± 57	2786 ± 452
	(0.25)	1344 ± 392	677 ± 160	3178 ± 452
P5	(25)	655 ± 116	331 ± 39	598 ± 61
	(2.5)	217 ± 44	184 ± 23	434 ± 53

* The stimulating antigens used were Med, medium control; PHA, phytohemagglutinin; TTxd, tetanus toxoid; BTxd, botulinum toxoid; P1-5, peptides 1-5 as in table 1.

\+ Each value represents mean ± 1 SEM, N=3. ND represents values not determined.

onto suitable carrier e.g. alhydrogel (alum) (not greater 0.15mg/ml aluminium, final concentration) in the presence of a preservative e.g. thimerisol (not more than 0.01% final concentration). Administration of one or more doses (0.5–1ml) is by various routes (e.g. intra-muscularly, sub-cutaneously) at various intervals. A vaccine can also be created by blending the absorbed peptide of the invention with existing botulinum vaccines.

Table 6

Helper T cell epitope maps to homologous regions of BTxd A, B, C and F

Stimulating Antigen (µg/ml)		[^{3}H]TdR incorporation** (CPM)	Stimulating Index
Expt.1			
Med		436 ± 101	-
P1	(5)	12948 ± 995	30
P2	(5)	227 ± 49	0.5
B5	(25)	4577 ± 383	11
	(5)	4099 ± 473	9.4
B6	(25)	2719 ± 615	6.2
B7	(5)	386 ± 70	0.9
Expt. 2			
Med		246 ± 101	-
TTxd	(0.1)	284 ± 57	1.2
BTxd A	(25)	5497 ± 370	22.3
	(5)	1663 ± 162	6.7
BTxd B	(25)	2070 ± 230	8.4
	(5)	816 ± 139	3.3
BTxd C	(5)	1148 ± 137	4.7
BTxd E	(25)	88 ± 12	0.4
	(5)	129 ± 5	0.5
BTxd F	(25)	1985 ± 49	8.0